

RE-EXAMINATION OF MUHLENBERGIA CAPILLARIS, M. EXPANSA,
AND M. SERICEA (POACEAE: MUHLENBERGIINAE)

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ABSTRACT

Molecular genetic data [intersimple sequence repeats (ISSR)] and morphological data support the recognition of *Muhlenbergia capillaris*, *M. expansa*, and *M. sericea* as separate species. Multi-response permutation analysis show significant differences ($T = -9.03$, $A = 0.20$, $P < 0.01$) among these three species indicating that individuals within a species were more genetically similar to one another than they were to individuals of another species. Apparently, *Muhlenbergia sericea* and *M. capillaris* are derived from a recent common ancestor, and are sister to *M. expansa*. A key to separate *Muhlenbergia capillaris*, *M. expansa*, and *M. sericea* is provided.

RESUMEN

Los datos genéticos moleculares [secuencias entre repeticiones simples (ISSR)] y los datos morfológicos apoyan el reconocimiento de *Muhlenbergia capillaris*, *M. expansa* y *M. sericea* como especies separadas. El análisis de permutación de respuesta múltiple ($T = -9.03$, $A = 0.20$, $P < 0.01$) entre estas tres especies que indican que los individuos dentro de una misma especie son genéticamente más similares entre si que entre los individuos de otras especies. Al parecer, *Muhlenbergia sericea* y *M. capillaris* son derivadas de un ancestro común reciente, y *M. expansa* es su especie hermana. Se proporciona una clave para separar *Muhlenbergia capillaris*, *M. expansa* y *M. sericea*.

Muhlenbergia Schreb. is primarily a Western Hemisphere genus of 152 species, with 69 species native to North America, north of México (Peterson 2003; Peterson et al. 2007). Members of this genus can be annual or perennial, rhizomatous to cespitose, and can occur in a variety of ecological settings. The genus is characterized by having solitary or rarely paired spikelets that are usually one-flowered; awned, mucronate or unawned lemmas that are three-veined; and a base chromosome number of $x = 10$ (Peterson et al. 1997). In recent years there has been some debate regarding the taxonomic status of *Muhlenbergia capillaris* (Lam.) Trin., *M. expansa* (Poir.) Trin., and *M. sericea* (Michx.) P.M. Peterson, three perennial cespitose species native to the southeastern and gulf coast of the United States, with the outcome potentially effecting cultural traditions and economic aspects of the Gullah peoples who have traditionally used *M. sericea* [synonyms: *Muhlenbergia filipes* M.A. Curtis; *Muhlenbergia capillaris* var. *filipes* (M.A. Curtis) Chapm. ex Beal] as the primary plant material to make sweetgrass baskets (Burke et al. 2003; Rosengarten 1986).

Muhlenbergia capillaris, *M. expansa* and *M. sericea* are all members of *Muhlenbergia* subgenus *Trichochloa* section *Podosemum* (Soderstrom 1967; Peterson & Herrera 2001). *Muhlenbergia sericea* is a perennial cespitose species that occurs in marginal maritime habitat along coastal barrier islands and woodlands of the southeastern and gulf coasts (TX to NC) and is characterized by long involute leaf blades (35–100 cm long), long-awned lemmas (8–35 mm long) and upper glumes (2–25 mm long), and lemmas with long setaceous teeth (1–5 mm long) near the apex. *Muhlenbergia capillaris* has a much wider ecological and geographical range (TX to KS to MA to FL) and superficially resembles *M. sericea*. However, *M. capillaris* has shorter-awned lemmas (2–18 mm long), unawned or shorter awned upper glumes (1–5 mm long), and usually lacks setaceous teeth or, if present, these are less than 1 mm long. *Muhlenbergia expansa* grows in wet pine savannas and pitcher plant flatwoods inland from the coastal plain (*M. sericea*) habitat and lacks awned upper glumes (sometimes these can be mucronate, i.e., with a mucro less than 1 mm long) or setaceous teeth. The lemmas of *M. expansa* are unawned, mucronate or have awns 1–3 mm long.

Morden and Hatch (1989) conducted a morphological study of *Muhlenbergia sericea*, *M. capillaris*, and

M. expansa specimens from 25 herbaria across the southeastern United States and suggested that these taxa are three varieties of *M. capillaris* rather than three distinct species. In our study, we use molecular genetic data [intersimple sequence repeats (ISSR)] to address the hypothesis that *Muhlenbergia sericea*, *M. capillaris*, and *M. expansa* are three distinct species. In addition, we offer a different interpretation of Morden and Hatch (1989) published data in support of our hypothesis that these are three distinct species.

METHODS

Plant genomic DNA was extracted from approximately 0.1 g of silica-dried leaves from field-collected plants, herbarium sheets, and 0.5 g fresh leaf material using E.Z.N.A.[®] plant DNA miniprep kit (Omega Bio-Tek, Doraville, Georgia, U.S.A.). *Muhlenbergia sericea* was collected from the eastern ($n = 8$, collected by K. Olandt in Charleston County, South Carolina) and western ($n = 4$, collected by P. Maywald in Kennedy County, Texas) range of the species in the fall of 2004. *Muhlenbergia expansa* DNA was extracted from two South Carolina herbarium sheets (*Townsend 2341 & 1123*) from the Clemson University Herbarium. *Muhlenbergia capillaris* extractions consisted of two individuals from Alabama (MacDonald 12080 & Allison 7225, University of Alabama Herbarium) and three individuals from South Carolina (collected in 2005 by DJG from Apron Island in Charleston County, South Carolina). Two individuals were extracted of *Muhlenbergia wrightii* Vasey ex J.M. Coult. and *M. montana* (Nutt.) Hitchc. grown from seeds that were purchased from Western Native Seed, Coaldale, Colorado.

Muhlenbergia montana and *M. wrightii* (outgroup) are perennial cespitose species native to the southwestern United States and occur on rocky slopes at elevations of 1100 to 3500 m. The former species has been included in the *Muhlenbergia montana* complex (Herrera 1998) and the later species is probably aligned with other genera in the Muhlenbergiinae (Peterson et al. 2004; Peterson et al. 2007).

Twenty five intersimple sequence repeat (ISSR) primers were surveyed, with six primers selected for this study (sequence, number of bands; (GT)₆-RG, 8 bands; (CA)₈-RG, 8 bands; (CA)₆-RY, 5 bands; (GA)₈-YC, 8 bands; (CT)₉-G, 3 bands; (CA)₆-RG, 4 bands). ISSR polymerase chain reaction (PCR) protocol followed that of Wolfe et al. (1998); 94° C for 1min 50sec, 40 cycles of 94° C for 40 sec, 43° C for 45 sec, and 72° C for 1min 50 sec, followed by a final extension at 72° C for 5 min. PCR profiles were visualized in 1.5% agarose gels and stained with ethidium bormide. Images were captured using a digital camera (Olympus C-4000 Zoom, Melville, NY), converted to a negative image, and fragment size was estimated based on a DNA marker (Benchtop pGEM, #G7521, Promega, Madison, WI). Fragment sizes were used to assign loci for each primer and bands were scored as diallelic for each locus (1=band present, 0= band absent). Individual ISSR profiles were used to calculate a priori species assignment using multi-response permutation procedure (MRPP) (PC-Ord, ver. 4.2, MjM Software Design, Gleneden Beach, Oregon, U.S.A.). Nei's genetic distance (1972) was calculated among taxa based on band frequency data and Neighbor-Joining cluster analysis (Saitou and Nei, 1987) using NTSYSpc 2.2d (NTSYSpc Numerical Taxonomy and Multivariate Analysis System, Applied Biostatistics Inc., New York, NY).

RESULTS AND DISCUSSION

ISSR analysis clearly supports *Muhlenbergia sericea* as a separate species that shares a common ancestor with *M. capillaris* and *M. expansa*, which is in agreement with Peterson's (2003) recent treatment of *Muhlenbergia*. MRPP analysis indicated significant differences ($T = -9.03$, $A = 0.20$, $P < 0.01$) among the a priori species designation, meaning that individuals within a species were more genetically similar to one another than they were to members of another species. If *Muhlenbergia sericea* and *M. expansa* were varieties of *M. capillaris*, then we would have expected to find members of all three a priori species forming one genetically similar grouping.

Phylogenetic relationships among these five *Muhlenbergia* species revealed predictable associations, with the more eastern *Muhlenbergia* species (*M. expansa*, *M. capillaris*, *M. sericea*) forming a monophyletic group (Fig. 1). *Muhlenbergia wrightii* (outgroup) and *M. montana* are native to the mountain and southwestern regions

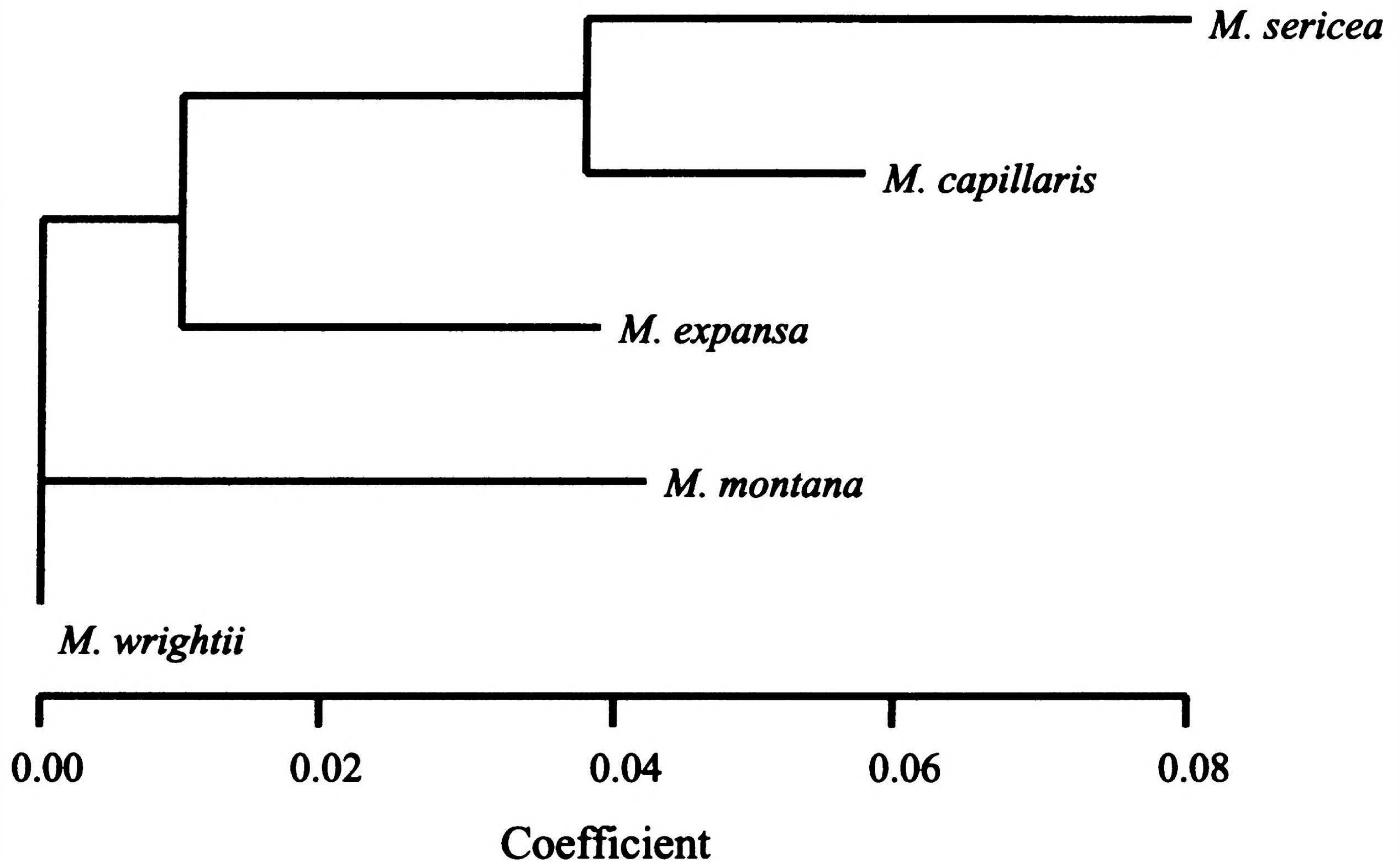


FIG. 1. Neighbor-Joining cluster analysis based on Nei's genetic distance among five *Muhlenbergia* species. The three southeastern *Muhlenbergia* species (*M. sericea*, *M. capillaris*, *M. expansa*) were more similar to one another than they were to the southwestern *M. montana* and mountain *M. wrightii* species.

of the United States and were clearly different from the three eastern species. Much of the taxonomic confusion surrounding *Muhlenbergia sericea*, *M. capillaris*, and *M. expansa* extends from the potential geographic and ecological overlap of these taxa and the limited number of diagnostic characters. Based on our ISSR molecular markers, *Muhlenbergia sericea* and *M. capillaris* are more similar to one another than they are to *M. expansa* (Fig. 1).

Morden and Hatch (1989) conducted a taxonomic study of *Muhlenbergia sericea*, *M. capillaris*, and *M. expansa* based on morphological characters and recommended that these three taxa should be a single species consisting of three varieties. We respectfully disagree with their conclusions and offer a different interpretation of their results. The taxonomic, geographic, and morphological sampling was sufficient and appropriate for the stated objectives of their study; however, they failed to use summary statistics to assess differences among taxa. An analysis of variance or non-parametric analysis should have yielded statistically significant differences among the three taxa for morphological characters which have been used historically to separate these species. Plotting the means \pm 1 standard error (Fig. 2) for blade, upper glume awn, lemma awn, and setaceous teeth lengths are a good indication that significant differences would have been found if the authors tested for difference among taxa. Morden and Hatch's PCA analysis does not present key multivariate statistics, such as eigenvalues for each axis or parallel analysis indicating which axes are appropriate for interpretation. In addition, a graph of individuals on the first two PCA axes clearly show *Muhlenbergia sericea* and *M. expansa* as separate clusters with *M. capillaris* intermediate (Fig. 1; Morden and Hatch 1989). Discriminate analysis statistics were also not presented in their manuscript; however, we would suggest that a misclassification rate of 3.7% (13 out of 350) is not strong support for realigning these three taxa as varieties of *Muhlenbergia capillaris*. In a draft (9 Aug 2006) of the Flora of the Carolinas, Georgia,

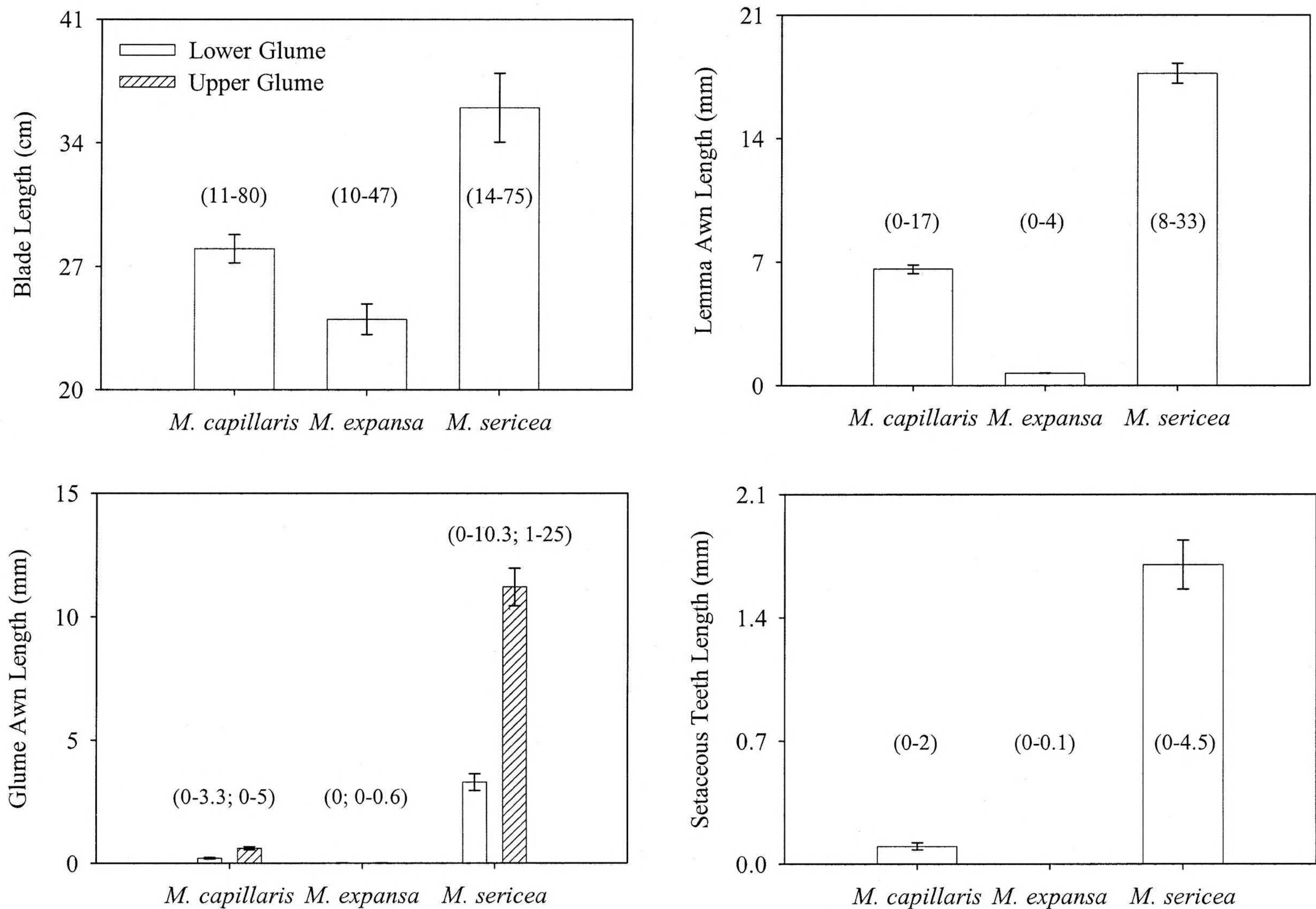


FIG. 2. These data represent the mean (\pm 1 SE), with range in parentheses, of key morphological characters used to distinguish *Muhlenbergia capillaris*, *M. expansa*, and *M. sericea*. Compiled from table 1 in Morden and Hatch 1989.

and Virginia, A.S. Weakley (in prep.) also comments in regards to Morden and Hatch (1989) and states that these three taxa are undoubtedly biological species.

Based on our molecular genetic data and a more rigorous statistical interpretation of Morden and Hatch (1989) morphological study, we conclude that *Muhlenbergia sericea* and *M. capillaris* are two closely related species that have limited ecological and morphological overlap, but these taxa should remain as distinct species. Morphological and ISSR analysis indicate that *Muhlenbergia sericea* and *M. capillaris* are more similar to one another than either is to *M. expansa*. While proper identification of *Muhlenbergia sericea*, *M. capillaris*, and *M. expansa* in the field is sometimes difficult, we recommend using a combination of ecological setting, blade length and mature floret characters (glume awn, lemma awn, and setaceous teeth length) to distinguish among these three closely related species. We provide a key to separate these three species below.

A KEY TO MUHLENBERGIA CAPILLARIS, M. EXPANSA, AND M. SERICEA IN NORTH AMERICA

1. Body of the glumes more than 1/2 as long as the lemmas; lemmas unawned, mucronate, or with awns only 1–3 mm long; upper glumes never awned but sometimes mucronate _____ **Muhlenbergia expansa**
1. Body of the glumes less than 1/2 as long as the lemmas; lemmas usually awned 2–35 mm long; upper glumes often awned, the awns 1–25 mm long.
 2. Upper glumes unawned or with awns to 5 mm long; lemmas without setaceous teeth or with teeth no more than 1 mm long; lemma awns 2–13(–18) mm long _____ **Muhlenbergia capillaris**
 2. Upper glumes awned, the awns 2–25 mm long; lemmas with setaceous teeth 1–5 mm long; lemma awns 8–35 mm long _____ **Muhlenbergia sericea**

ACKNOWLEDGMENTS

We thank Patricia Gomez Bustamante for help preparing the Spanish resumen. We would also like to thank the University of Alabama, University of Florida, and Clemson University Herbaria for providing pressed material and Paula Maywald for material from Texas. We acknowledge the thoughtful comments and suggestions by Drs. Mark Basinger and Barney Lipscomb which improved this manuscript. We also acknowledge the contributions made by the Citadel Plant Ecology Laboratory undergraduate researchers (Philip Strole, Esteban Sierra, Chris Morrow, and Garret Ryan). This research was funded by grants from the South Carolina Sea Grant Consortium and The Citadel Foundation to DJG.

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